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Microbac Protocol

Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection *Trichophyton interdigitale*

Testing Facility
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Prepared for
STERIS CORPORATION
7405 Page Avenue
St. Louis, MO 63133-1032

June 21, 2017

Microbac Protocol: 429.2a.06.21.17
Microbac Project No.: 429 - 392

OBJECTIVE:

This test is designed to substantiate disinfectant effectiveness for impregnated or pre-saturated towelettes, single or multiple uses, to be registered with the Environmental Protection Agency and Health Canada. The test evaluates the effectiveness of products as disinfectants for contaminated surfaces. The test follows the "Germicidal Spray Products as Disinfectants" test as described in the Official Methods of Analysis, Eighteenth Edition, 2012, AOAC and the EPA Notice of Efficacy Requirements for Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection. This test also meets the EPA OCSPP 810.2000 and 810.2200 Product Performance Test Guidelines and Health Canada "Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs" as applicable.

TESTING CONDITIONS:

Using a single test substance (1), per lot (2), per microorganism (1), ten carriers (10), per contact time (1) for a total of 20 replicates will be evaluated. Carriers inoculated with *Trichophyton interdigitale* will be wiped as directed by the sponsor or label instruction and held for the exposure time and at the temperature specified by the sponsor. The carriers will be cultured, incubated and observed for visible growth.

MATERIALS:

A. Test, control and reference substances will be supplied by the sponsor of the study (see last page). As per CFR 40.160.105:

- The identity, strength, purity, and composition, or other characteristics which will appropriately define the test, control, or reference substance shall be determined for each lot and shall be documented by the sponsor before its use in a study. Methods of synthesis, fabrication, or derivation of the test, control, or reference substance shall be documented and retained by the sponsor.

- When relevant to the conduct of the study the solubility of each test, control, or reference substance shall be determined by the sponsor before the experimental start date. The stability of the test, control, or reference substance shall be determined by the sponsor before the experimental start date or concomitantly according to written standard operating procedures, which provide for periodic analysis of each lot.

The test substance will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the test substance such as dilution or specialized storage conditions must be specified by the sponsor before initiation of testing.

The sponsor assures Microbac Laboratories, Inc. (Microbac) testing facility management that the test substance has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

Microbac will retain all unused test substances for a period of at least one year after completion of the test, and then discard them in a manner that meets the approval of the safety officer.

B. Materials supplied by Microbac, including, but not limited to:

1. Challenge microorganism, required by the sponsor of the study: *Trichophyton interdigitale*, ATCC 9533 (TI)

2. Media and reagents:
   a. Sterile saline (SS)
   b. Suitable agar media: Neopeptone Glucose Agar (NGA)
   c. Neutralizer: Recovery broth with required neutralizer(s)
   d. Letheen Broth (LB)
   e. Heat-inactivated fetal bovine serum (if required)
   f. Phosphate Buffered Dilution Water (PBDW)

3. Laboratory equipment and supplies, including glass microscope slides (1" x 3" with a 1" x 1" surface for contamination and treatment)

TEST SYSTEM IDENTIFICATION:

As applicable, all test and control tube racks will be labeled with microorganism, test substance, lot identifier, and project number prior to initiation of the study and during incubation. Petri dishes will be labeled with microorganism prior to initiation of the study and microorganism and project number during incubation. Test substance and usage will be traced according to SOPs currently existing in the laboratory.

EXPERIMENTAL DESIGN:

A. Inoculum preparation:

The fungus will be inoculated from stock cultures onto agar plates and incubated at 25 - 30C for ≥ 10, but ≤ 15 days or until sporulation. When the cultures appear to be mature, the mycelial mats will be removed from the surface of at least five plates and macerated with SS in a tissue grinder. The suspension will be filtered through sterile glass wool to remove the hyphae. The density of the conidial suspensions will be determined by standard plate count techniques. The plates will be incubated for 3-5 days at 25 - 30C. The suspension will be stored at 2 - 10C for ≤ 4 weeks before use. If requested by the sponsor, heat-inactivated fetal bovine serum will be added to the inoculum to yield a final concentration of 5% on the day of the test.

B. Carrier preparation and inoculation:

The new carriers will be visually screened and discarded if visibly damaged (scratched, chipped or nicked). The carriers will be rinsed with 95% ethanol followed by a rinse with deionized water to remove oil and film on the slides. The carriers will be sterilized by placing in evaporating dishes matted with two pieces of filter paper, heating them in a hot air oven for two hours at 180C, cooling and storing them at room temperature until use.

Using a positive displacement pipet, a 0.01 mL (10 µL) aliquot of each culture will be transferred onto a one-square inch area on the sterile carriers (in Petri dishes) and immediately spread uniformly over the entire area with a sterile glass rod. Each dish will be covered promptly and the operation will be repeated for the rest of the carriers. Carriers will be dried for 30-40 minutes at 36±1C. The humidity level of the incubator during the drying phase required for the inoculated carriers will be monitored and reported. Inoculated carriers will be used for testing within two hours of drying.

C. Test substance preparation:

Immediately before testing, pressure will be applied to the pouch containing the wipes and the liquid will rupture the bladder to saturate the wipes. The test substance will be allowed to equilibrate to ambient room temperature for a least 10 minutes before testing.

D. Test:

Note: The temperature and humidity level of the laboratory during the test phase will be monitored and reported.

Ten carriers will be evaluated whereas a single wipe will be used to treat each set of 10 carriers based on the following:

Each towelette will be folded in such a way that one towelette will be used to treat ten carriers. Each carrier (maintained in the Petri dish that was used for carrier inoculation) will be wiped for the specified time requested by the sponsor. The area of the towelette used for wiping will be rotated to expose a new, unused surface for each carrier, allowing a maximum surface area of the towelette to be used over the course of the procedure.

Initially the towelette will be folded lengthwise twice and then folded five times inward beginning from the far end. Then the outside edges will be pulled upward to form a "U" shape and grasped on one side with the thumb and on the other side with the index and middle finger.

The initial contaminated carrier (for each 10 replicate set) will be wiped using two complete horizontal strokes, with one right to left and back to right considered as one stroke; and then wiped using two complete vertical strokes, with one up to down and back to up considered one stroke for a total of four complete strokes.

After the treatment of the initial carrier for the 10 replicate set, the area of the towelette used for wiping will be rotated to expose a new, unused surface for each carrier while allowing a maximum surface area of the towelette to be used over the course of the procedure.

The used end will be flipped upward towards itself, reoriented appropriately and then used to wipe the next carrier. The next three carriers will be wiped in a similar fashion whereas the used portion will be folded up-and-over each time.

Once five carriers have been wiped, the second lengthwise fold will be unfolded and refolded in the opposite direction. The towelette will be refolded five times in the same manner as the original pre-folded state and the above procedure for wiping the first five carriers will be repeated for wiping the last five carriers. This process will be repeated until a total of ten carriers have been wiped with one wipe.

Once treated, each carrier will be held in a horizontal position for the exposure time as specified by the sponsor. After the contact period, each carrier will be transferred to tubes containing 20 mL of the Neutralizer using sterile forceps within the ±5 sec. (or ±3 sec.) time limit and shaken thoroughly. For products with ≤1 minute contact time, the transfer will be made within ±3 seconds. The slide can touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible.

All tubes will be incubated at 25-30C for up to ten days and the results recorded as visible growth or no visible growth.

E. Controls:

1. Sterility controls:

   One sterile carrier will be added to a tube of Neutralizer and incubated with the test to demonstrate the sterility of the media used in the study.

2. Viability controls:

   Two inoculated carriers will be independently transferred into tubes Neutralizer and incubated with the test to serve as comparison for the test cultures.

3. Neutralizer effectiveness:

   Using sterile two carriers, per lot, the test substance will be applied to the carriers, held for the contact time and subcultured into individual tubes of Neutralizer based on the procedures used for the test (with the exception that two replicates, per test substance will be treated).

   To each tube, fewer than 100 colony forming units (CFU) of the challenge microorganism will be added and the count of the fungi inoculated into these tubes will be confirmed in duplicate suitable agar media spread plates. The tubes will be incubated with the test. The plates will be incubated for 3-5 days at 25-30C.

4. Carrier counts:

   The average CFU per carrier will be determined using three inoculated carriers, per lot immediately after the conclusion of the processing of the test replicates for a total of six replicates.

   Dried inoculated carriers will be placed individually into tubes containing 20 mL LB. The tubes will be immediately vortexed for 120±5 seconds. After vortexing, serial ten-fold dilutions of each suspension will be performed in PBDW blanks. Duplicate one mL aliquots from selected dilutions will be plated in suitable agar media spread plates. Diluting and plating will be completed within 2 hours after vortexing. All plates will be incubated for 3-5 days at 25-30C and the average CFU/carrier determined.

5. Confirmation of challenge microorganism:

   Following incubation, at least 20% of the test tubes exhibiting growth and all viability tubes will be streaked onto suitable agar media plates and incubated for 3-5 days at 25-30C. Wet mounts will be performed on all viability tubes and all of the colonies that appear to be the challenge microorganism from the test streaks. Preparations will be compared to that of a viability control tube.

PRODUCT EVALUATION CRITERIA:

According to the EPA, the test substance passes the test if no visible growth is observed any of the test tubes (0/10) per lot and the controls meet their stipulated criteria. There is no statistical method proposed for this protocol.

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- The average of the carrier counts must be at least 1.0×10^4 CFU/carrier.
- The $\log_{10}$ density (LD) for each carrier will be determined based on the following:
  - Dilutions yielding counts up to 300 CFU will be used.
  - Plate counts of 0 will be included in the calculations.
  - The CFU/mL (of broth) will be calculated:

$$\text{CFU/mL} = \frac{(\text{avg. CFU for } 10^{-x}) + (\text{avg. CFU for } 10^{-y}) + (\text{avg. CFU for } 10^{-z})}{10^{-x} + 10^{-y} + 10^{-z}}$$

  - The CFU/carrier will be calculated by multiplying the CFU/mL by the volume of broth into which the fungi were harvested from the carrier by vortex-mixing (20 mL).
  - The LD for each carrier will be calculated by taking the Log_{10} of the density (per carrier).
- The recovery broth with neutralizers must be proven effective
- The sterility control must be negative for growth
- The viability control must be positive for growth
- The neutralization confirmation tubes must show growth following inoculation with <100 CFU per tube to confirm effective neutralization.
- The purity of the challenge microorganism must be confirmed based on the procedures employed for confirmation

DATA PRESENTATION:

The final report will include the following information:

- The number of positive carriers per test substance (or lot).
- The average colony-forming units per carrier.
- The results of all controls.

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned before initiation of the test. Resumes for technical personnel are maintained and are available on request. This study will be conducted at Microbac, 105 Carpenter Drive, Sterling, VA 20164.

REGULATORY COMPLIANCE AND QUALITY ASSURANCE (GLP studies only):

This study will be performed in compliance with the US Environmental Protection Agency's Good Laboratory Practices (GLP) regulations, 40 CFR 160 (note: information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test substance resides with the sponsor of the study unless otherwise stated).

The Quality Assurance Unit of Microbac will inspect the conduct of the study for GLP compliance. The dates of the inspections and the dates that findings are reported to the study management and study director will be included in the final report.

PROTOCOL AMENDMENTS AND DEVIATIONS:

Any protocol amendment(s) and protocol deviation(s) identified will be reported in project sheet(s) and included in the final report.

REPORT FORMAT:

The report will contain all items required by EPA 810.2200 and be in compliance with EPA PR Notice 2011-3 (replaced PRN 86-5). Microbac employs a standard report format for each test design. Each final report will provide at least the following information:

- Sponsor identification
- Test substance identification
- Type of assay and project number
- Study start and end time (clock time)
- Interpretation of results and conclusions
- Test results presented in tabular form
- Methods and evaluation criteria, if applicable
- Dates of study initiation and completion (GLP studies only)
- Signed Quality Assurance and Compliance Statements (GLP studies only)

RECORDS TO BE MAINTAINED:

For all GLP studies, the original signed final report will be sent to the Sponsor.

A draft report will be provided to Sponsor for review prior to finalization of the report. All raw data, protocol, protocol modifications, test substance records, copy of final report, and correspondence between Microbac and the sponsor will be stored in the archives at Microbac, 105 Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test substance; challenge microorganism used and the type of neutralizers employed in the test will be addressed in a project sheet issued separately for each study. The date the study director signs the protocol will be the initiation date. All project sheets issued will be forwarded to the study sponsor for appropriate action.

MISCELLANEOUS INFORMATION: The following information is to be completed by sponsor before initiation of study:

A. Name and address: STERIS CORPORATION
7405 Page Avenue
St. Louis, MO 63133-1032

B. Test substance information:

Test substance name	EXP16042	
Lot No.	Lot No. 1	Lot No. 2
	PFR2566A	PRF2566B
Active ingredient(s)	PAA, H_2O_2	

C. Test Conditions:

Preparation	See Page 4, Section C for activation details		
Diluent	Not applicable		
Lower Certified Limit (LCL)	■ yes	☐ No	☐ Not applicable
Contact time	9.5 minutes		
Contact temperature	Ambient Room Temperature (20±1C)		

D. Organic load – serum (HI FBS) added to achieve 5% in the inoculum: ■ yes ☐ no

E. Precautions/storage conditions: MSDS and/or C of A provided: ■ yes ☐ no

REPORT HANDLING AND STUDY CONDUCT: EPA & Health Canada, GLP

PROTOCOL APPROVAL:

Sponsor Signature: _____ Date: 8/22/17

Dan Klein, STERIS CORPORATION

Study Director Signature: _____ Date: 08/23/17

Rana Rahmat, Microbac